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Selectivity and Mode of Action of Carfentrazone-ethyl, a Novel Phenyl Triazolinone Herbicide

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Abstract: Post-emergence application of carfentrazone-ethyl at rates as low as 2·2 g ha^{-1} caused greater leaf injury and growth reduction in ivyleaf morningglory (*Ipomoea hederacea*) and velvetleaf (*Abutilon theophrasti*) than in soybean (*Glycine max*). The herbicide was more rapidly metabolized in the crop than in the weed species, with 26·7, 54·3 and 60·6% of the parent compound remaining in soybean, ivyleaf morningglory and velvetleaf, respectively, 24 h after exposure. The free acid metabolite, carfentrazone, was present in all species and accounted for 21·2–27·4% of the total radioactivity. Unknown metabolites (R_f 0 and 0·22) were four to five times more abundant in soybean than in the weed species. Carfentrazone-ethyl induced more leakage from leaf discs from the weeds than those from soybean and the degree of injury correlated with the amount of protoporphyrin IX (Proto IX) present in the treated tissues. Both carfentrazone-ethyl and carfentrazone were potent inhibitors of protoporphyrinogen oxidase (Protox). Therefore, the selectivity of this herbicide may, at least in part, be attributed to the lower accumulation of Proto IX in soybean than in the weeds, probably because of the ability of soybean to metabolize more carfentrazone into unknown metabolites than the weeds.

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1 INTRODUCTION

Carfentrazone-ethyl, (ethyl 2-chloro-4-[2-chloro-4-fluoro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]phenyl]propionate; F-8426; **1**), is an experimental post-emergence herbicide of the phenyl triazolinone group currently being developed by the FMC Corporation (Agricultural Products Group, Philadelphia, PA 19103, USA). This molecule is structurally similar to, and more active than sulfentrazone (**3**), which is the first herbicide of this class to be commercialized.[1]

Carfentrazone-ethyl is herbicidally active against morningglories (*Ipomoea* spp.) and velvetleaf (*Abutilon theophrasti* Medicus) that pose serious problems in soybean (*Glycine max* (L.) Merr.) fields of the southern United States.[2] Competition between soybean and velvetleaf may result in as much as 46% yield reduction,[3] and infestation by morningglories can reduce soybean yields by 75%.[4]

Little information is available on phenyl triazolinones. The mode of action of sulfentrazone, the only phenyl triazolinone previously studied in this class,

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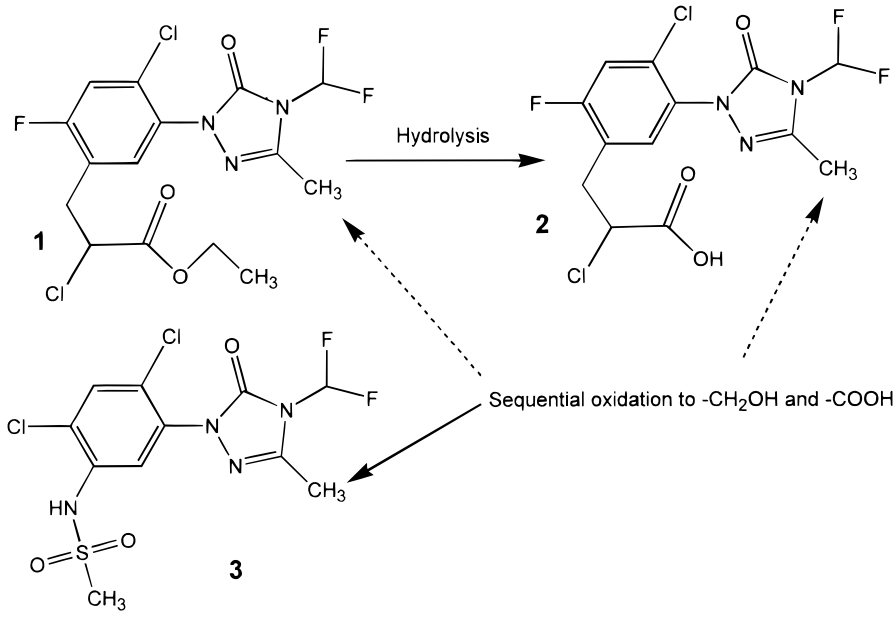

Fig. 1. Structures of phenyl triazolinones cited in the text: **1**, carfentrazone-ethyl; **2**, carfentrazone; **3**, sulfentrazone. The solid arrow between **1** and **2** indicates the hydrolytic degradation of carfentrazone-ethyl. The bottom arrow to **3** indicates the known site of oxidative degradation of sulfentrazone. The dashed arrows indicate the possible sites for similar oxidative degradation of carfentrazone-ethyl.

is the inhibition of protoporphyrinogen oxidase (Protox),[5,6] which causes the accumulation of protoporphyrin IX (Proto IX)[7–10] that is involved in the light-dependent formation of singlet oxygen responsible for membrane peroxidation.[11]

While injury to the foliage of carfentrazone-treated plants is similar to that caused by other Protox-inhibiting herbicides (e.g. photobleaching and necrosis of the foliage), the exact mode of action of this phenyl triazolinone herbicide has not been established.

The purpose of this study was to determine the herbicidal activity of carfentrazone-ethyl on soybean cv. 'Hutcheson', velvetleaf and ivyleaf morningglory (*Ipomoea hederacea* (L.) Jacq.), and the basis for selectivity of this herbicide. We also report information pertaining to the molecular target of this herbicide and the formation of a biologically active metabolite.

2 MATERIALS AND METHODS

2.1 Herbicides and analytical grade standards

Carfentrazone-ethyl 500 g kg^{-1} WG and technical grade analytical standards were provided by the FMC Corporation. [*U*-^{14}C-*phenyl*] carfentrazone-ethyl (**1**) had a radiochemical purity greater than 96% and specific activity of $1{\cdot}4 \times 10^9$ Bq $mmol^{-1}$.

2.2 Post-emergence activity of formulated carfentrazone

Seeds were planted 2·5 cm deep in 10 × 10 cm plastic pots in commercial substrate (PRO-MIX, Premier Brand Inc., Red Hill, PA 18076, USA). Aqueous dispersions of carfentrazone-ethyl WG were applied to 14-day-old plants using a 8001 flat fan nozzle (Teejet flat-fan tips, Spraying Systems Co., Wheaton, IL 60188, USA) and a moving-belt sprayer calibrated to deliver 1450 litre ha^{-1} at rates of 0, 2·2 and 9 g AI ha^{-1}. A surfactant, X-77, (2·5 ml $litre^{-1}$) was incorporated in the spray liquid.

Plants were maintained in greenhouse under natural light conditions and watered daily. Temperature and relative humidity ranged from 23 to 34°C and 60 to 96%, respectively. Plant height was measured and the foliage was rated for phytotoxicity at 7 and 14 days after treatment (DAT). Ratings ranged from 0 to 100% foliar injury. A rating of 100 was assigned to plants with severe necrotic foliage and/or complete defoliation.[12] One replication consisted of eight pots with four seeds per pots for each species. This experiment was arranged in a completely randomized design with three replications and repeated three times.

2.3 Metabolic fate of carfentrazone-ethyl

2.3.1 Application of radioactive carfentrazone-ethyl

A 10 μl droplet containing $1{\cdot}2 \times 10^6$ Bq [^{14}C]carfentrazone-ethyl and 2·5 ml $litre^{-1}$ X-77 was applied to the adaxial surface of leaves of 14-day-old plants. The growth stage of the treated leaves at the time of treatment was the first trifoliate leaf for soybean and the third leaf for ivyleaf morningglory and velvetleaf. One leaf or leaflet per plant was treated with the radiolabeled solution. The entire treated leaf or leaflets were collected after 24 h exposure to the radioactivity, and the

unabsorbed radioactivity was removed from the surface of the leaves by washing them with methanol + water (1 + 1 by volume). The percentage of radioactivity absorbed was calculated by subtracting the amount of radioactivity recovered from the wash from the total amount of radioactivity applied. A total of six leaves were treated and analyzed separately for each species. Treated tissues were kept at −80°C prior to extraction.

2.3.2 Extraction of the carfentrazone-ethyl and metabolites

Treated tissues were pulverized in liquid nitrogen and radioactivity was extracted with methanol + water (9 + 1 by volume; 10 ml). Plant debris was collected by centrifugation for 10 min at 3000 g and washed twice more as described above. The combined extracts were evaporated to dryness under vacuum and redissolved in methanol (5 ml).

2.3.3 Analysis of plant extracts

The radioactive components present in the extracts were separated by thin layer chromatography (TLC) on 20 × 20 cm plates coated with silica gel 60A (250 μm thickness) using hexane + ethyl acetate + acetic acid (30 + 90 + 1 by volume) as the solvent system. The position of analytical standards of carfentrazone-ethyl and the free acid form, carfentrazone (2-chloro-4-[2-chloro-4-fluoro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1-*H*-1,2,4-triazol-1-yl]phenyl]propionic acid, **2**) was visualized under UV light (254 nm) after spraying the plates with Rhodamine 6-G (5 g litre^{-1} in methanol); their R_f values were 0·68 and 0·55, respectively.

The distribution of radioactivity on chromatograms was analyzed with an imaging scanner (Bioscan 200, Bioscan Inc. Washington, DC, USA). Data for the metabolism experiment represent the pooled average of two replications with three observations each.

2.4 Determination of herbicide-induced cellular damage

Foliar tissues of soybean, ivyleaf morningglory and velvetleaf were treated with 10 μM technical grade carfentrazone-ethyl or carfentrazone by cutting 50 4 mm leaf discs (approximately 0·2 g of fresh weight) and placing them in a 6 cm diameter disposable Petri dish in 5 ml of a solution containing 10 g litre^{-1} sucrose and 1 mM 2-(*N*-morpholino)ethanesulfonic acid; (MES, pH 6·5) with or without the test compound.[13] All test compounds were dissolved in methanol. Controls contained the same amount of methanol without the test compounds. The final concentration of methanol in the Petri dishes was 10 ml litre^{-1}.

Tissues were incubated at 25°C in darkness for 20 h and then exposed to 500 μmol m^{-2} s^{-1} PAR for 24 h. Cellular damage was measured as electrolyte leakage into the bathing medium using a conductivity meter with the capability of assaying 1 ml of bathing medium and returning it to the dish.[13] Conductivity was monitored for 20 h in darkness, followed by 24 h of continuous light. Because of differences in background conductivity of treatment solutions, results were expressed as change in conductivity after initial measurement at the beginning of the dark period. The maximum conductivity was determined for each species by boiling the samples 30 min and measuring conductivity after 24 h. All treatments for electrolyte leakage measurements were triplicated and repeated twice. Representative electrolyte leakage data from two experiments are presented in Fig. 2 because of differences in absolute values between the two experiments conducted. However, the relative differences in response to carfentrazone between species were similar.

2.5 Protox preparation and determination of I_{50} values

Crude etioplast preparations were obtained from the cotyledonary tissues of 10-day-old dark-grown soybean seedlings according to the method of Sherman *et al.*[10] Procedures for protoporphyrinogen (Protogen) preparation and Protox assay were according to Jacobs and Jacobs.[14] The enzyme assay mixture consisted of *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES; 100 mM), ethylenediamine tetraacetic acid (EDTA; 5 mM), DTT (2 mM) and 20 μl of substrate. The I_{50} values (concentration of herbicide required to obtain 50% inhibition of the enzyme) were determined under saturated substrate conditions (2 mM Protogen) in the presence of 0, 1, 10, 100, 1000 and 10 000 nM technical grade carfentrazone-ethyl or the free acid. The reaction was initiated by adding 100 μl of the etioplast suspension, which had been pre-incubated with or without herbicide for 15 min on ice prior to the assay,

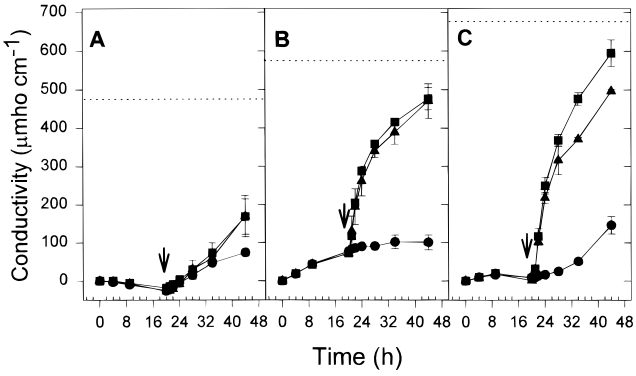

Fig. 2. Herbicidal activity of carfentrazone-ethyl as measured by electrolyte leakage from (A) leaf discs of soybean, (B) velvetleaf and (C) ivyleaf morningglory. Leaf discs were incubated in the presence of 10 μM carfentrazone-ethyl or free acid metabolite for 20 h in darkness and then exposed to continuous light. (●) control; (■) carfentrazone-ethyl (▲) free acid; arrows indicate start of light exposure and dotted lines indicate maximum conductivity from boiled samples.

TABLE 1
Foliar Injury to Soybean and Selected Weeds in Response to Post-Emergence Application of Carfentrazone-ethyl

Species	*Rate*[a] ($g\ ha^{-1}$)	*Foliar injury*[b]				
		0 DAT	*7 DAT* (%)($\pm SD$)		*14 DAT*	
Soybean	0	0	0	a	0	a
	2·2	0	37 ($\pm$10)	b	19 ($\pm$6)	b
	9	0	51 ($\pm$6)	c	36 ($\pm$6)	c
Ivyleaf morningglory	0	0	0	a	0	a
	2·2	0	94 ($\pm$10)	b	92 ($\pm$13)	b
	9	0	100	b	98 ($\pm$13)	b
Velvetleaf	0	0	0	a	0	a
	2·2	0	100	b	98 ($\pm$3)	b
	9	0	100	b	100	b

[a] Applied with 2·5 g $litre^{-1}$ X-77.
[b] For each species, numbers within a column followed by same letters are not different at $P < 0{\cdot}05$, according to Fischer's protected LSD.

to 900 μl of assay mixture, and carried out for 1 min at 30°C. Fluorescence was recorded using a luminescence spectrometer (RF-5000U, Shimadzu, Japan) with excitation and emission wavelengths set at 395 and 626 nm, respectively. The excitation and emission bandwidths were set at 1·5 and 30 nm, respectively. All assays were triplicated and the experiment was repeated.

2.6 Competitive binding of the herbicide to its molecular target site

Binding of [^{14}C]acifluorfen (AF) to soybean etioplasts in the presence or absence of carfentrazone-ethyl was determined according to the method of Tischer and Strotmann.[15] Etioplasts (0·6 mg of protein) were suspended in a reaction solution containing sorbitol (330 mM), HEPES (pH 7·7; 100 mM), EDTA (1 mM) and $MgCl_2$ (1 mM). Various concentrations (2–100 nM) of [^{14}C]-labeled AF (specific activity 18·03 mCi $mmol^{-1}$, uniformly ring labeled) plus 100 nM of carfentrazone-ethyl was added. The suspensions were mixed and incubated for 30 min on ice. The samples were centrifuged for 6 min at 12 000 g at 4°C. The supernatant was transferred to vials and mixed with 12 ml of premixed scintillation cocktail (Ecolume, ICN, Costa Mesa, CA 92626, USA) for radioactivity measurements. The inner walls of tubes were wiped dry with cotton swabs without disturbing the pellets to remove any adhering [^{14}C]. A 100 μl aliquot of tissue solubilizer (Protosol, NEN, Boston, MA 02118, USA) was added to pellets and heated in a water bath at 50°C for 15 min. The slurry was neutralized with tris(hydroxymethyl) aminomethane hydrochloride (TRIS-HCl, pH 7·0; 1 M; 50 μl) and transferred to vials. The inner walls of tubes were washed with 50 μl ethanol and added to the slurry before radioactivity measurements.

The amount of [^{14}C]AF bound was calculated from the radioactivity in the pellets. Specific binding constants were estimated from double-reciprocal plots of bound AF versus free AF.[15] For example, extrapolation of the regression lines of [^{14}C]AF binding to the x axis (shown in Fig. 4) gave a value of 0·049 065 1/nM or 20·4 nM, which is the specific binding constant to AF. The binding of each compound was triplicated and the experiment was repeated.

2.7 Determination of cellular concentration of Proto IX

2.7.1 Extraction of Proto IX

All extractions of Proto IX from seedling leaf discs were made under dim, green light source after 20 h incubation in the presence of 10 μM technical grade carfentrazone-ethyl in darkness at 25°C. Samples (approximately 0·1 g of soybean, velvetleaf and ivyleaf morningglory leaf discs) were homogenized in HPLC grade methanol + 0·1 M NH_4OH (9 + 1 by volume; 2 ml) with a Brinkman Polytron homogenizer at full speed for 15 s. The homogenates were washed with hexane (4 × 2 ml) and centrifuged at 6000 g for 10 min at 4°C. The supernatants were filtered through a 0·2 μm nylon syringe filter and stored in light-tight glass vials at −20°C until analysis by HPLC.

2.7.2 Analysis of Proto IX content

The HPLC system was composed of Waters Associates, (Milford, MA 01757, USA) components which included a Model 510 pump, a Model 712 autosampler, a Millennium 2010 controller, and Models 470 fluorescence and 990 photodiode spectrophotometric detectors. The column was a 250 × 4·6 mm (ID). Spherisorb 5 μm ODS-1 reversed-phase preceded by a Bio-Rad ODS-5S guard column. The solvent system consisted of 80%

HPLC-grade methanol with 30% tetrabutylammonium phosphate (PIC A) reagent. The injection volume was 50 μl. The position of Proto IX in chromatograms was determined using a Proto IX standard (Sigma Chemical Co., St. Louis, MO 63178, USA). Proto IX detection was performed with fluorescence detector with excitation and emission wavelength settings at 400 and 630 nm, respectively, and the peaks were confirmed by scanning them from 300 to 700 nm with the photodiode array detector. The data are expressed on a molar basis per g fresh weight. All treatments were triplicated.

2.8 Statistical analysis

Statistical analyses were done with SAS, and the means were tested by Fischer's protected LSD at $P < 0{\cdot}05$. The means in Table 1 were arc sine transformed before being tested by Fischer's protected LSD.

3 RESULTS AND DISCUSSION

3.1 Post-emergence activity of carfentrazone-ethyl on soybean, velvetleaf and ivyleaf morningglory

3.1.1 Foliar injury

Carfentrazone-ethyl applied at as low as 2·2 g ha^{-1} caused severe injury to ivyleaf morningglory (94%) and velvetleaf (100%) at 7 DAT (Table 1). During the same period, both weed species were killed when the herbicide rate was increased to 9 g ha^{-1}. Weeds did not overcome the phytotoxic effects of carfentrazone-ethyl with 92 and 98% injury at 2·2 g ha^{-1} and 98 and 100% at 9 g ha^{-1}, at 14 DAT (Table 1).

Soybean was more tolerant than the weeds to carfentrazone-ethyl. The foliage of soybean exhibited 37 and 51% injury following 2·2 and 9 g ha^{-1} application, respectively, at 7 DAT (Table 1). Furthermore, soybean seedlings began to recover from the herbicide-induced injury by 14 DAT, with ratings of 19 and 36%, respectively (Table 1). Symptoms included bronzing, achlorophyllous veins, and some necrosis, and were similar to those observed with sulfentrazone[12] and other photobleaching herbicides.[13,16–20]

3.1.2 Effect of carfentrazone-ethyl on shoot height

The extremely rapid foliar damage observed in the weeds was accompanied by significant growth reduction. Plant heights of the carfentrazone-ethyl-treated plants were 84 and 99% less than those of untreated controls for ivyleaf morningglory, and 96 and 100% for velvetleaf, at seven days after application of 2·2 and 9 g ha^{-1}, respectively (Table 2). Stand reduction remained the same at 14 DAT, indicating that these weed species were unable to overcome the injury caused by the herbicide (Table 2). Although soybean seedlings exhibited some foliar injury, growth of carfentrazone-ethyl-treated plants was not different from that of controls (Table 2). Therefore, this herbicide had selectivity and did not cause stand reduction to the soybean while controlling the weeds.

3.2 Metabolic fate of carfentrazone-ethyl

Metabolic degradation is the most common mechanism of natural resistance to Protox inhibitors. Frear *et al.*[21] have shown that soybean resistance to acifluorfen is associated with cleavage of the ether bond and conjugation of the metabolites. More recently, we have shown that soybean rapidly metabolized sulfentrazone[5] and that the metabolites were less phytotoxic than the parent.[22]

In this study, carfentrazone-ethyl was more readily metabolized by soybean than by the weed species. Only

TABLE 2
Growth Response of Soybean and Selected Weeds to Post-Emergence Application of Carfentrazone-ethyl

Species	*Rate*[a] (*g ha*$^{-1}$)	*Height*[b] (*cm*) (±*SD*)		
		0 DAT	*7 DAT*	*14 DAT*
Soybean	0	12·0 (±1·9) a	15·8 (±2·6) a	18·3 (±4·0) a
	2·2	14·1 (±0·9) a	18·1 (±0·4) a	21·7 (±1·4) a
	9	13·9 (±2·4) a	15·5 (±1·6) a	17·4 (±2·8) a
Ivyleaf morningglory	0	12·0 (±6·1) a	15·0 (±0·1) a	27·9 (±5·4) a
	2·2	14·1 (±3·4) a	2·4 (±3·7) b	3·2 (±5·6) b
	9	12·0 (±2·7) a	0·2 (±0·3) b	0·2 (±0·3) b
Velvetleaf	0	6·9 (±1·1) a	9·2 (±0·7) a	12·6 (±1·4) a
	2·2	6·9 (±1·2) a	0·4 (±0·4) b	0·3 (±0·2) b
	9	6·8 (±1·2) a	0 b	0 b

[a] Applied with 2·5 g litre^{-1} X-77.
[b] For each species, numbers within a column followed by same letters are not different at $P < 0{\cdot}05$, according to Fischer's protected LSD.

26·7% of the parent molecule remained in the treated tissue of soybean after 24 h exposure, while 54·3 and 60·6% remained in ivyleaf morningglory and velvetleaf, respectively (Table 3). All species studied hydrolysed carfentrazone-ethyl (**1**) to the free acid derivative, carfentrazone (**2**). This metabolite accounted for 21·2, 20·1 and 27·4% of the total radioactivity remaining in soybean, velvetleaf and ivyleaf morningglory, respectively, after 24 h exposure (Table 3).

At least two unidentified metabolites were observed in each of the species studied. Unknown 1 (R_f 0·22) accounted for 37·5% of the total radioactivity extracted from soybean, but only 6·9 and 9·1% from ivyleaf morningglory and velvetleaf (Table 3). Unknown(s) 2 remaining at the origin on the TLC plates accounted for 18·5, 14·5 and 5·8% of the radioactivity in ivyleaf morningglory, soybean and velvetleaf, respectively (Table 3). The inability of unknown(s) 2 to migrate on the TLC plate in the solvent system used suggests the presence of polar metabolites, perhaps in conjugated forms. Less than 5% of the radioactivity remained in the post-extraction cellular debris indicating that metabolites were not readily incorporated into cell wall components during the 24-h period of the experiment.

It is clear that the metabolism of carfentrazone-ethyl to forms other than the free acid, and particularly to the unknown 1, occurs more readily in soybean than in the weeds used in this study. The identities of the unknown metabolites remain to be determined. However, oxidative degradation of the methyl substituent on the triazolinone ring of sulfentrazone is involved in the detoxification of this herbicide in sicklepod (*Senna obtusifolia* (L) Irwin & Barneby)[23] and soybean (**3**).[5] Such hydroxylation, often associated with P-450 monooxygenase activity,[24,25] may occur with carfentrazone-ethyl (Fig. 1).

While soybean apparently metabolized carfentrazone-ethyl more rapidly than did the weed species included in this study, our data indicate that significant amounts of the herbicide remain even in the tissues of soybean. Furthermore, all the species accumulated substantial amounts of the free acid metabolite. We were, therefore, interested in determining the herbicidal activity of the free acid metabolite relative to that of the parent compound.

3.3 Effects of carfentrazone-ethyl and the free acid metabolite on cellular leakage

Species variation in sensitivity to carfentrazone-ethyl was also evident in a leaf-disc assay which measured the leakage of electrolytes from herbicide-treated leaf tissues as indicated by increased electrical conductivity in the bathing solution. Electrolyte leakage was greater in the weeds than in soybean. Conductivity in the bathing medium after 24 h light exposure was 83 and 88% of the maximum conductivity for velvetleaf and morningglory, respectively, while it was only 35% for soybean (Fig. 2). This difference in species sensitivity is probably associated with the variation in metabolism we reported above. Photobleaching of the tissues was evident in the weeds after 8 h light exposure, but was not detected in soybean during the entire duration of the experiment. Loss of membrane integrity exhibited by the weeds is consistent with symptoms associated with other Protox inhibitors.[5,13]

Interestingly, the free acid metabolite of carfentrazone-ethyl was also toxic to the weeds in the leaf-disc assay (Fig. 2), and raises the question as to the exact nature of the active molecule, i.e. is carfentrazone-ethyl the active ingredient or is it a metabolically activated proherbicide? To answer this question, we determined the site of action and in-vitro activities of these two compounds.

3.4 In-vitro activity of carfentrazone-ethyl and the free acid metabolite

Both carfentrazone-ethyl and the free acid metabolite inhibited Protox and, in that regard, are similar to sulfentrazone and the diphenyl ether herbicides. However, their I_{50} values were 18 and 6 nM, respectively, which is about 50 to 100 times more potent than sulfentrazone (Fig. 3) and 20 times more than acifluorfen.[26] In agreement with studies on other Protox-inhibiting herbicides, the level of inhibition is correlated with the binding

TABLE 3
Metabolic Fate of [^{14}C]Carfentrazone-ethyl in Soybean and Selected Weed Species after 24 h Exposure

Species	*Absorbed*	*Carfentrazone-ethyl and metabolites*			
		Parent molecule	*Free acid*	*Unknown 1*	*Unknown 2*
	(%)(±*SD*)	(%)(±*SD*)			
Soybean	54 (±5)	26·7 (±2·5)	21·2 (±5·9)	37·5 (±5·0)	14·5 (±2·7)
Ivyleaf morningglory	49 (±7)	54·3 (±9·5)	20·1 (±4·2)	6·9 (±2·1)	18·7 (±6·7)
Velvetleaf	66 (±3)	60·6 (±7·2)	27·4 (±5·9)	9·1 (±4·7)	5·8 (±4·4)

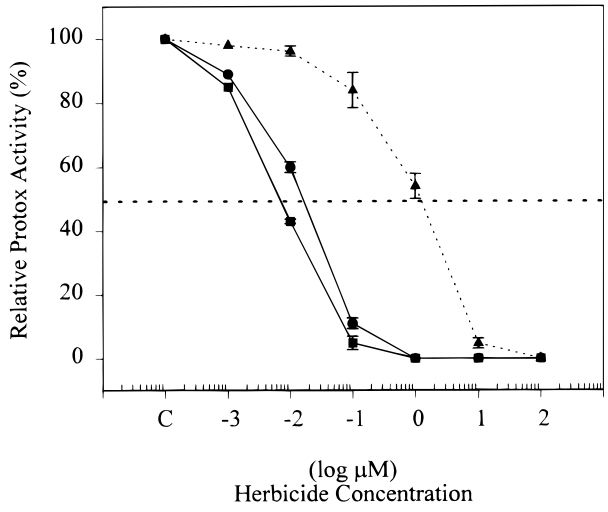

Fig. 3. Protox inhibition by phenyl triazolinones. (●) carfentrazone-ethyl (■) free acid. (▲) sulfentrazone, shown with dotted line for comparison of activity between phenyl triazolinones.

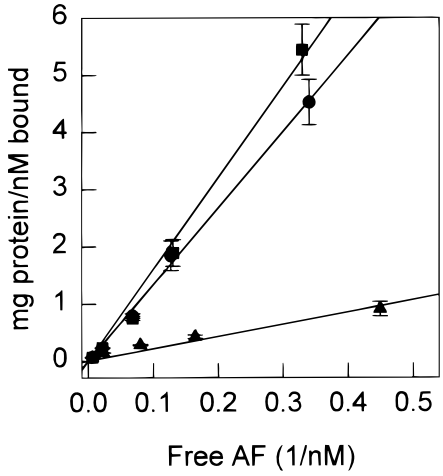

Fig. 4. Binding of labeled acifluorfen (AF) in the presence of 100 nM unlabeled carfentrazone-ethyl or its free acid derivative. (●) carfentrazone-ethyl (■) free acid derivative; (▲) acifluorfen alone.

affinity of the herbicide at or near the catalytic site on Protox (Fig. 4).[11,26–31]

Both carfentrazone-ethyl and the free acid had high binding constants (258 and 285 nM, respectively) and readily displaced radiolabeled AF from its binding site on Protox (Fig. 4). Although these compounds were potent inhibitors of soybean Protox, they induced little growth reduction to soybean in the greenhouse nor any substantial damage in the leaf-disc assays. Therefore, resistance mechanisms other than low binding affinity and differential metabolic degradation may participate in the selectivity of this herbicide in soybean.

It has been established by others[7,20] that the activity of photobleaching herbicides is correlated with the amount of Proto IX accumulating in treated tissues. We determined that little Proto IX accumulated in soybean exposed to either carfentrazone-ethyl or the free acid (Table 4). The lack of accumulation of Proto IX may be due to the combined effect of metabolism and of a recently discovered enzymatic activity that degrades Protogen.[32] More Proto IX accumulated in the tissues of ivyleaf morningglory and the highest level was found in velvetleaf (Table 4). This latter weed was also the most sensitive to post-emergence application of carfentrazone-ethyl (Tables 1 and 2).

4 CONCLUSIONS

Carfentrazone-ethyl is a selective post-emergence herbicide against representative problem weeds in soybean fields. The hydrolysis of the ethyl ester cannot be considered a detoxifying step in the metabolism of the herbicide since the free acid metabolite strongly inhibits Protox. However, the ability of soybean to further metabolize carfentrazone-ethyl, particularly to form unknown 1, may play a role in the tolerance of this crop, relative to the weeds. Nevertheless, phytotoxic forms of the compound, e.g. ethyl ester (**1**) and free acid (**2**), accumulate in the tissues of all species studied, indicating that other factors may protect plants against photodynamic damage, such as higher endogenous levels of antioxidants[33] or oxygen radical scavenging enzyme systems,[34–36] and rapid enzymatic degradation

TABLE 4
Effect of 10 μM Carfentrazone-ethyl or the Free Acid derivative on Proto IX Accumulation in Leaf Discs of Soybean, Velvetleaf and Ivyleaf Morningglory after 20 h Incubation in Darkness

	Carfentrazone-ethyl		*Free acid*	
Species	*Control*	*Treated*	*Control*	*Treated*
	(*nmol Proto IX* g^{-1} *fresh weight*)[a]			
Soybean	0·02 a	0·42 c	0·01 a	0·41 c
Velvetleaf	0·01 a	8·38 a	0·03 a	7·63 a
Ivyleaf morningglory	0·01 a	1·15 b	0·01 a	1·22 b

[a] Numbers within a column followed by same letters are not different at $P < 0{\cdot}05$, according to Fischer's protected LSD.

of Proto IX or Protogen IX[32] may also participate in the selectivity of carfentrazone-ethyl.

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